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More priorities on the following pages

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solution)

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Remarks:

**THE COMPLETE DOCUMENT INCLUDING  
REFERENCE TABLES AND THE SEQUENCE  
LISTING IS AVAILABLE ON CD-ROM FROM THE  
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(54) **Sequence-determined DNA fragments and corresponding polypeptides encoded thereby**

(57) The present invention provides DNA molecules that constitute fragments of the genome of a plant, and polypeptides encoded thereby. The DNA molecules are useful for specifying a gene product in cells, either as a promoter or as a protein coding sequence or as an UTR or as a 3' termination sequence, and are also useful in controlling the behavior of a gene in the chromosome,

in controlling the expression of a gene or as tools for genetic mapping, recognizing or isolating identical or related DNA fragments, or identification of a particular individual organism, or for clustering of a group of organisms with a common trait.

<sup>0</sup>Arabidopsis DNA is used in the present experiment, but the procedure is a general one.

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(30) Priority: Continued from first pag

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[2347] The suspension culture cells are transformed with exogenous DNA as described by Z. Chen et al. *Plant Mol. Bio.* 36:163 (1998). Briefly, 4-days post-subculture cells are incubated with cell wall digestion solution containing 0.4 M sorbitol, 2% driselase, 5mM MES (2-[N-Morpholino] ethanesulfonic acid) pH 5.0 for 5 hours. The digested cells are pelleted gently at 60 xg for 5 min. and washed twice in W5 solution containing 154 mM NaCl, 5 mM KCl, 125 mM CaCl<sub>2</sub> and 5mM glucose, pH 6.0. The protoplasts are suspended in MC solution containing 5 mM MES, 20 mM CaCl<sub>2</sub>, 0.5 M mannitol, pH 5.7 and the protoplast density is adjusted to about 4 x 10<sup>6</sup> protoplasts per ml.

[2348] 15-60 µg of plasmid DNA is mixed with 0.9 ml of protoplasts. The resulting suspension is mixed with 40% polyethylene glycol (MW 8000, PEG 8000), by gentle inversion a few times at room temperature for 5 to 25 min. Protoplast culture medium known in the art is added into the PEG-DNA-protoplast mixture. Protoplasts are incubated in the culture medium for 24 hours to 5 days and cell extracts can be used for assay of transient expression of the introduced gene. Alternatively, transformed cells can be used to produce transgenic callus, which in turn can be used to produce transgenic plants, by methods known in the art. See, for example, Nomura and Komamine, *Pit. Phys.* 79: 988-991 (1985), *Identification and Isolation of Single Cells that Produce Somatic Embryos in Carrot Suspension Cultures*.

[2349] The invention being thus described, it will be apparent to one of ordinary skill in the art that various modifications of the materials and methods for practicing the invention can be made. Such modifications are to be considered within the scope of the invention as defined by the following claims.

[2350] Each of the references from the patent and periodical literature cited herein is hereby expressly incorporated in its entirety by such citation.

## Claims

1. An isolated nucleic acid molecule comprising a nucleic acid having a nucleotide sequence which encodes an amino acid sequence exhibiting at least 40% sequence identity to an amino acid sequence encoded by

- (a) a nucleotide sequence described in REF and/or SEQ Table 1 or 2 or a fragment thereof; or
- (b) a complement of a nucleotide sequence shown in REF and/or SEQ Table 1 or 2 or a fragment thereof.

2. An isolated nucleic acid molecule comprising a nucleic acid having a nucleotide sequence which exhibits at least 65% sequence identity to

- (a) a nucleotide sequence shown in REF and/or SEQ Table 1 or 2 or a fragment thereof; or
- (b) a complement of a nucleotide sequence shown in REF and/or SEQ Table 1 or 2 or a fragment thereof.

3. An isolated nucleic acid molecule comprising a nucleic acid having a nucleotide sequence which exhibits at least 65% sequence identity to a gene comprising

- (a) a nucleotide sequence shown in REF and/or SEQ Table 1 or 2 or a fragment thereof; or
- (b) a complement of a nucleotide sequence shown in REF and/or SEQ Table 1 or 2 or a fragment thereof.

4. An isolated nucleic acid molecule which is the reverse of the isolated nucleotide sequence according to any one of claims 1-3, such that the reverse nucleotide sequence has a sequence order which is the reverse of the sequence order of said isolated nucleotide sequence according to any one of claims 1-3.

5. An isolated nucleic acid molecule comprising a nucleic acid capable of hybridizing to a nucleic acid having a sequence selected from the group consisting of:

- (a) a nucleotide sequence which is shown in REF and/or SEQ Table 1 or 2; and
- (b) a nucleotide sequence which is complementary to a nucleotide sequence shown in REF and/or SEQ Table 1 or 2;

under conditions that permit formation of a nucleic acid duplex at a temperature from about 40°C and 48°C below the melting temperature of the nucleic acid duplex.

6. The nucleic acid molecule according to any one of claims 1-5, wherein said nucleic acid comprises an open reading frame.

7. The isolated nucleic acid molecule of any one of claims 1-5, wherein said nucleic acid is capable of functioning as a promoter, a 3' end termination sequence, an untranslated region (UTR), or as a regulatory sequence.
- 5 8. The isolated nucleic acid molecule of claim 7, wherein said nucleic acid is a promoter and comprises a sequence selected from the group consisting of a TATA box sequence, a CAAT box sequence, a motif of GCAATCG or any transcriptoin-factor binding sequence, and any combination thereof.
9. The isolated nucleic acid molecule of claim 7, wherein the nucleic acid sequence is a regulatory sequence which is capable of promoting seed-specific expression, embryo-specific expression, ovule-specific expression, tapetum-specific expression or root-specific expression of a sequence or any combination thereof.
- 10 10. A vector construct comprising a nucleic acid molecule according to any one of claims 1-9, wherein said nucleic acid molecule is heterologous to any element in said vector construct.
- 15 11. A vector construct according to claim 10 comprising:
  - (a) a first nucleic acid having a regulatory sequence capable of causing transcription and/or translation; and
  - (b) a second nucleic acid having the sequence of said isolated nucleic acid molecule according to any one of claims 1-4;
- 20 wherein said first and second nucleic acids are operably linked and wherein said second nucleic acid is heterologous to any element in said vector construct.
12. The vector construct according to claim 11, wherein said first nucleic acid is native to said second nucleic acid.
- 25 13. The vector construct according to claim 11, wherein said first nucleic acid is heterologous to said second nucleic acid.
14. A vector construct according to claim 10 comprising:
  - (c) a first nucleic acid having having the sequence of said isolated nucleic acid molecule according to claim 7; and
  - (d) a second nucleic acid;
- 30 wherein said first and second nucleic acids are operably linked and wherein said first nucleic acid is heterologous to any element in said vector construct.
15. The vector construct according to claim 14, wherein said first nucleic acid is native to said second nucleic acid.
- 40 16. The vector construct according to claim 14, wherein said first nucleic acid is heterologous to said second nucleic acid.
17. A host cell comprising an isolated nucleic acid molecule according to any one of claims 1-4, wherein said nucleic acid molecule is flanked by exogenous sequence.
- 45 18. A host cell comprising a vector construct of any one of claims 10-16.
19. An isolated polypeptide comprising an amino acid sequence
  - (a) exhibiting at least 40% sequence identity of an amino acid sequence encoded by a sequence shown in REF and/or SEQ Table 1 or 2 or a fragment thereof; and
  - (b) capable of exhibiting at least one of the biological activities of the polypeptide encoded by said nucleotide sequence shown in REF and/or SEQ Table 1 or 2 or a fragment thereof.
- 50 20. The isolated polypeptide of claim 19, wherein said amino acid sequence exhibits at least 75% sequence identity to an amino acid sequence encoded by a sequence shown in SEQ Table 1 or 2 or a fragment thereof.
- 55 21. The isolated polypeptide of claim 19, wherein said amino acid sequence exhibits at least 85% sequence identity

to an amino acid sequence encoded by a sequence shown in SEQ Table 1 or 2 or a fragment thereof.

22. The isolated polypeptide of claim 19, wherein said amino acid sequence exhibits at least 90% sequence identity to an amino acid sequence encoded by a sequence shown in SEQ Table 1 or 2 or a fragment thereof.
23. An antibody capable of binding the isolated polypeptide of any one of claims 19-22.
24. A method of introducing an isolated nucleic acid into a host cell comprising:
  - (a) providing an isolated nucleic acid molecule according to any one of claims 1-4; and
  - (b) contacting said isolated nucleic acid with said host cell under conditions that permit insertion of said nucleic acid into said host cell.
25. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to any one of claims 10-16.
26. A method of modulating transcription and/or translation of a nucleic acid in a host cell comprising:
  - (a) providing the host cell of claim 24 or 25; and
  - (b) culturing said host cell under conditions that permit transcription or translation.
27. A method for detecting a nucleic acid in a sample which comprises:
  - (a) providing an isolated nucleic acid molecule according to any one of claims 1-5;
  - (b) contacting said isolated nucleic acid molecule with a sample under conditions which permit a comparison of the sequence of said isolated nucleic acid molecule with the sequence of DNA in said sample; and
  - (c) analyzing the result of said comparison.
28. The method according to claim 27, wherein said isolated nucleic acid molecule and said sample are contacted under conditions which permit the formation of a duplex between complementary nucleic acid sequences.
29. A plant or cell of a plant which comprises a nucleic acid molecule according to any one of claims 1-4 which is exogenous to said plant or plant cell.
30. A plant or cell of a plant which comprises a nucleic acid molecule according to any one of claims 1-4, wherein said nucleic acid molecule is heterologous to said plant or said cell of a plant.
31. A plant or cell of a plant which has been transformed with a nucleic acid molecule according to any one of claims 1-4.
32. A plant or cell of a plant which comprises a vector construct according to any one of claims 10-16.
33. A plant or cell of a plant which has been transformed with a vector construct according to any one of claims 10-16.
34. A plant which has been regenerated from a plant cell according to any one of claims 29-33.

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Max Len. Seq. :

rel to:

Clone IDs:

34671

(Ac) cDNA SEQ

- Pat. Appln. SEQ ID NO: 28401
- Ceres SEQ ID NO: 1571425

PolyP SEQ

- Pat. Appln. SEQ ID NO 28402
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(C) Pred. PP Nom. & Annot.

(Dp) Rel. AA SEQ

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<210> 28402

<211> 385

<212> PRT

<213> Arabidopsis thaliana

<220>

<223> any n or Xaa = unknown

<220>

<223> Location 1..385 / Ceres Seq. ID 1571426

<400> 28402

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20          25          30
Lys Arg Ile Ser Glu Val Val Val Phe Lys Gly Tyr Asp Arg Gly Asp
35          40          45
Tyr Val Val Arg Glu Asn Gln Asn Val Asp Gly Val Tyr Phe Leu Leu
50          55          60
Gln Gly Gln Ala Gln Val Leu Arg Ser Ala Glu Glu Glu Asn Tyr Gln
65          70          75          80
Glu Phe Pro Leu Lys Arg Tyr Asp Phe Phe Gly His Gly Ile Phe Gly
85          90          95
Asp Val Tyr Ser Ala Asp Val Val Ala Val Thr Glu Leu Thr Cys Leu
100          105          110
Leu Leu Met Ser Asp His Arg Ala Leu Leu Glu Ile Lys Ser Val Ser
115          120          125
Asp Ser Asp Lys Glu Arg Cys Leu Val Glu Asp Ile Leu Tyr Leu Glu
130          135          140
Pro Leu Asp Leu Asn Val Tyr Arg Gly Phe Thr Pro Pro Asn Ala Pro
145          150          155          160
Thr Tyr Gly Lys Val Tyr Gly Gly Gln Leu Val Gly Gln Ala Leu Ala
165          170          175
Ala Ala Ser Lys Thr Val Glu Thr Met Lys Ile Val His Asn Phe His
180          185          190
Cys Tyr Phe Leu Leu Val Gly Asp Ile Asn Ile Pro Ile Ile Tyr Asp
195          200          205
Val Asn Arg Leu Arg Asp Gly Asn Asn Phe Ala Thr Arg Ser Val Asp
210          215          220
Ala Arg Gln Lys Gly Lys Thr Ile Phe Thr Leu Phe Ala Ser Phe Gln
225          230          235          240
Lys Lys Gln Gln Gly Phe Ile His Gln Glu Ser Thr Met Pro His Thr
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<213> Arabidopsis thaliana

<223> any n or Xaa = unknown

<223> Location 1..377 / Ceres Seq. ID 1571427

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Phe	Lys	Gly 35	Tyr	Asp	Arg	Gly	Asp 40	Tyr	Val	Val	Arg	Glu 45	Asn	Gln	Asn
Val	Asp 50	Gly	Val	Tyr	Phe	Leu 55	Leu	Gln	Gly	Gln	Ala 60	Gln	Val	Leu	Arg
Ser 65	Ala	Glu	Glu	Glu	Asn 70	Tyr	Gln	Glu	Phe	Pro 75	Leu	Lys	Arg	Tyr	Asp 80
Phe	Phe	Gly	His	Gly 85	Ile	Phe	Gly	Asp	Val 90	Tyr	Ser	Ala	Asp	Val 95	Val
Ala	Val	Thr	Glu 100	Leu	Thr	Cys	Leu	Leu 105	Leu	Met	Ser	Asp	His 110	Arg	Ala
Leu	Leu	Glu 115	Ile	Lys	Ser	Val	Ser 120	Asp	Ser	Asp	Lys	Glu 125	Arg	Cys	Leu
Val	Glu 130	Asp	Ile	Leu	Tyr	Leu 135	Glu	Pro	Leu	Asp	Leu 140	Asn	Val	Tyr	Arg
Gly 145	Phe	Thr	Pro	Pro	Asn 150	Ala	Pro	Thr	Tyr	Gly 155	Lys	Val	Tyr	Gly	Gly 160
Gln	Leu	Val	Gly	Gln 165	Ala	Leu	Ala	Ala 170	Ala	Ser	Lys	Thr	Val	Glu 175	Thr
Met	Lys	Ile	Val 180	His	Asn	Phe	His	Cys 185	Tyr	Phe	Leu	Leu	Val 190	Gly	Asp
Ile	Asn 195	Ile	Pro	Ile	Ile	Tyr	Asp 200	Val	Asn	Arg	Leu	Arg	Asp	Gly	Asn
Asn	Phe 210	Ala	Thr	Arg	Ser	Val 215	Asp	Ala	Arg	Gln	Lys 220	Gly	Lys	Thr	Ile

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Phe Thr Leu Phe Ala Ser Phe Gln Lys Lys Gln Gln Gly Phe Ile His
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Gln Glu Ser Thr Met Pro His Thr Pro Ala Pro Glu Thr Leu Leu Pro
                245                250                255
Arg Glu Glu Met Leu Glu Arg Leu Val Thr Glu Pro Leu Leu Pro Arg
                260                265                270
Asp Tyr Arg Asn Gln Val Ala Thr Glu Ile Ser Val Pro Phe Pro Ile
                275                280                285
Asp Ile Arg Phe Cys Glu Pro Asn Arg Ser Thr Lys Gln Asn Lys Ser
                290                295                300
Pro Pro Arg Leu Lys Tyr Trp Phe Arg Ala Lys Gly Lys Leu Ser Asp
305                310                315                320
Asp Asp Gln Ala Leu His Arg Cys Val Val Ala Phe Ala Ser Asp Leu
                325                330                335
Ile Phe Ala Thr Ile Ser Leu Asn Pro His Arg Arg Glu Gly Met Ser
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Val Ala Ala Leu Ser Leu Asp His Ser Met Trp Phe His Arg Pro Val
                355                360                365
Arg Ala Asp Asp Trp Leu Leu Phe Val
370                375

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&lt;210&gt; 28404

&lt;211&gt; 271

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

&lt;220&gt;

&lt;223&gt; any n or Xaa = unknown

&lt;220&gt;

&lt;223&gt; Location 1..271 / Ceres Seq. ID 1571428

&lt;400&gt; 28404

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Asp Leu Asn Val Tyr Arg Gly Phe Thr Pro Pro Asn Ala Pro Thr Tyr
                35                40                45
Gly Lys Val Tyr Gly Gly Gln Leu Val Gly Gln Ala Leu Ala Ala Ala
50                55                60
Ser Lys Thr Val Glu Thr Met Lys Ile Val His Asn Phe His Cys Tyr
65                70                75                80
Phe Leu Leu Val Gly Asp Ile Asn Ile Pro Ile Ile Tyr Asp Val Asn
                85                90                95
Arg Leu Arg Asp Gly Asn Asn Phe Ala Thr Arg Ser Val Asp Ala Arg
                100                105                110
Gln Lys Gly Lys Thr Ile Phe Thr Leu Phe Ala Ser Phe Gln Lys Lys
                115                120                125
Gln Gln Gly Phe Ile His Gln Glu Ser Thr Met Pro His Thr Pro Ala
130                135                140
Pro Glu Thr Leu Leu Pro Arg Glu Glu Met Leu Glu Arg Leu Val Thr
145                150                155                160
Glu Pro Leu Leu Pro Arg Asp Tyr Arg Asn Gln Val Ala Thr Glu Ile
                165                170                175
Ser Val Pro Phe Pro Ile Asp Ile Arg Phe Cys Glu Pro Asn Arg Ser
180                185                190
Thr Lys Gln Asn Lys Ser Pro Pro Arg Leu Lys Tyr Trp Phe Arg Ala
195                200                205

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Lys Gly Lys Leu Ser Asp Asp Asp Gln Ala Leu His Arg Cys Val Val  
 210 215 220  
 Ala Phe Ala Ser Asp Leu Ile Phe Ala Thr Ile Ser Leu Asn Pro His  
 225 230 235 240  
 Arg Arg Glu Gly Met Ser Val Ala Ala Leu Ser Leu Asp His Ser Met  
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 Trp Phe His Arg Pro Val Arg Ala Asp Asp Trp Leu Leu Phe Val  
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<210> 28405

<211> 1974

<212> DNA

<213> *Arabidopsis thaliana*

<220>

<223> any n or Xaa = unknown

<220>

<223> Location 1..1974 / Ceres Seq. ID 1571432

<400> 28405

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aaagttgaca	gcttggttagg	gaagaagatt	ggattgtatt	tttcagctgc	ttggtgtgga	240
ccgtgtcagc	ggtttactcc	acagctgggtg	gaagtctaca	acgagctctc	ttcgaaaagt	300
ggttttgaga	ttgtgtttgt	gtcaggtgat	gaggatgaag	agtcatttgg	agattatttc	360
agtaagatgc	cctggctcgc	tgttccgttt	actgattcgg	aaacccgtga	ccgtttggat	420
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ccagagaaaa	tgaaggagat	caaagaggat	gaagatagag	ctcggagaga	acagacctta	600
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<210> 28406

<211> 470

<212> PRT

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